STUDIES ON DESIGN, SYNTHESIS, CHARACTERIZATION OF NOVEL HETEROCYCLIC COMPOUNDS AND THEIR PHARMACOLOGICAL ACTIVITES

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Introduciton

The heterocycles are so ubiquitous due to their plethora of biological process and wide distributions in nature. In chemical terms, these are generally cyclic structures with at least one non-carbon atom typically nitrogen, oxygen and sulfur. They play a vital role in the metabolism of all living things, and are utilized at almost every stage of the many biochemical processes necessary to sustain life. These are the molecules that have the ability to bind with variety of enzymes in multitude ways such as hydrogen bonding, pi stacking interactions, metal co-ordination bonds, van der Waals interaction, hydrophobic forces etc. In addition, their wide range of ring sizes and structural permutations means heterocycles come in a broad range of shapes and sizes, allowing them to match the equally diverse structural range of enzyme binding pockets [1]. With their functional versatility, extremely common occurrence in nature, and involvement in large numbers of biological pathway this work is focused on synthesis and biological evaluation of some of the heterocyclic compounds to justify their place in the race to combating problems in biomedical field.

Amidst all the azole-fused heterocycles, we have chosen imidazopyridine as a lead molecule. It has been recognized as drug prejudice [3] scaffold with its remarkable biological activities as it bioisosteres with the indoles and azaindoles. Furthermore, its stride in pharmaceutical industry as anticancer, antiviral, antiprotozoal, antipyretic, anti inflammatory, analgesic etc are remarkable. Due to all these therapeutic values, the core structure of imidazopyridine is found in some of the established drugs such as zolpidem (hypnotic drug), fadrazole (antineoplastic), alpidem, saripiden and necopidem (anxiolytic), miroprofen (analgesics), olprinone (cardiotonic agents), rifaximin (antibiotic), zolimidine (antiulcer), minodornic acid (treatment of osteoporosis) GSK812397 (CXCR4antagonist) etc [4-5].

Among the plethora of heterocyclic nucleus discovered, the oxadiazoles have been explored extensively. The oxadiazole structure has been demonstrated to bear important biological activities such as anti-cancer, anti-inflammatory, anti-tuberculosis, anti-malarial and anti-microbial etc. The presence of oxadiazole motifs in diverse types of compounds proves its importance in the field of medicinal chemistry. In some cases, they have been used as an essential part of the pharmacophore, favorably contributing to ligand binding. In other cases, oxadiazole moieties have shown to act as an aromatic linker to place substituent in appropriate orientation, and modulating molecular properties by positioning them in the periphery of the

molecule. Oxadiazoles have been used as replacements for carbonyl containing compounds such as esters, amides, carbamates and hydroxamic esters [6].

Triazole ring system has got considerable fame due to the versatile biological activities of a huge number of its derivatives. Among the heterocyclic compounds, triazoles are one of the most key heterocycles exhibiting remarkable pharmacological activity as they are an essential constituent of all cells and living matter. Triazole is a five-membered heterocyclic ring, which possesses three nitrogen atoms. The parent compound, triazole was synthesized for the first time by Fischer in 1878. Over 0.2 million triazole derivatives have been reported in the literature and this class of organic compounds has become extraordinarily important due to their wide-range of biological activity as antitumor, antiviral, antibacterial and also in the field of agro-chemicals [7].

Chalcones are an encompassing plethora of natural and synthetic compounds. The functionalities of this α, β-unsaturated carbonyl system has shown broad spectrum of biological activities including anti-inflammatory, antioxidant, antitumor, antidiabetic, antimicrobial etc [8]. They remain popular as scaffolds because of their structural simplicity. Not only they are abundantly found in nature, but also are key intermediates for many other medicinally important heterocyclic compounds. Being electrophilic in nature, several therapeutically important heterocyclic molecules can be synthesized such as pyrroles, indoles, isoxazoles, imidazoles, pyrazoles, indazoles, triazoles, pyridines and pyramidines [9].

Pyrazoles are reported to possess a wide range of biological activities in literature such as anti-microbial, anti-fungal, anti-tubercular, anti-inflammatory, anti-convulsant, anticancer, anti-viral, angiotensin converting enzyme (ACE) inhibitor, neuroprotective, cholecystokinin-1 receptor antagonist, and estrogen receptor (ER) ligand activity, etc [10].Many pyrazole derivatives have already found their application asdrugs, such as anti-pyrine or phenazone (analgesic and antipyretic), metamizole or dipyrone (analgesic and antipyretic), aminopyrine or aminophenazone (anti-inflammatory, antipyretic, and analgesic), phenylbutazone (anti-inflammatory, antipyretic mainly used in osteoarthritis, rheumatoid arthritis, spondylitis, Reiter's disease), sulfinpyrazone (chronic gout), and oxyphenbutazone (antipyretic, analgesic, anti-inflammatory, mild uricosuric) etc [11].

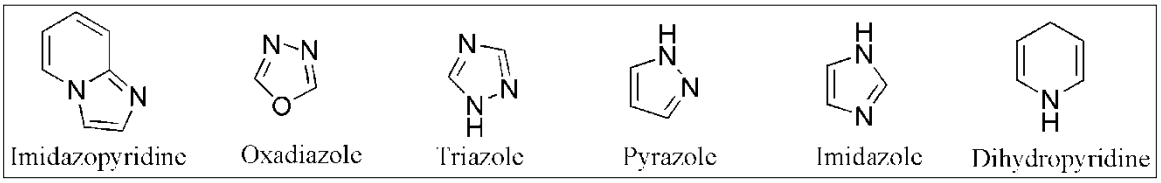
The 1, 4-dihydropyridines (DHPs), a class of drugs possesses a wide variety of biological and pharmacological actions. It represents one of the most important groups of calcium-channel modulating agents and has experienced widespread use in the treatment of cardiovascular disease

which includes antihypertensive, vasodilator and cardiac depressants activities. It also shows antibacterial, anticancer, antileishmanial, anticoagulant, anticonvulsant, antitubercular, antioxidant, antiulcer, antimalarial, neuroprotection properties, HIV-1 protease inhibitors, antifertility activities and many more. There are many drugs available in market which contains 1, 4-dihydropyridine ring as basic scaffold [12].

Imidazole nucleus forms the main structure of some well-known components of human organisms, that is, the amino acid histidine, Vit-B12, a component of DNA base structure and purines, histamine, and biotin. It is also present in the structure of many natural or synthetic drug molecules, that is, cimetidine, azomycin, and metronidazole. Imidazole-containing drugs have a broader scope in remedying various dispositions in clinical medicine [13].

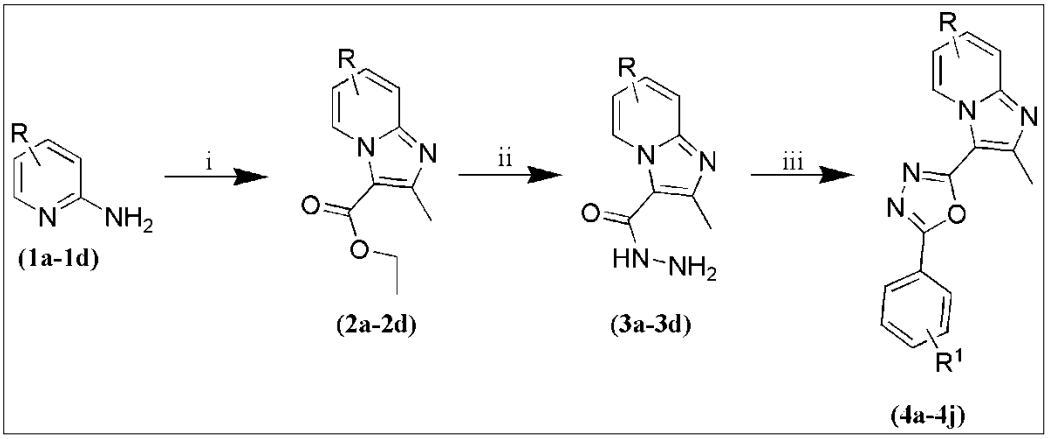
Chapter 1: A brief review on some heterocyclic compounds.

This is an introductory chapter, which deals with the importance of different heterocyclic compounds. This chapter mainly focuses on the brief literature survey of imidazopyridine, triazole, chalcones, pyrazoles, pyridines and imidazoles; comprising their synthetic routes and corresponding biological applications.



Chapter 2: Synthesis, characterization, single crystal XRD, Hirshfeld surface and antimicrobial activities of some new imidazopyridine derivatives incorporated with oxadiazoles.

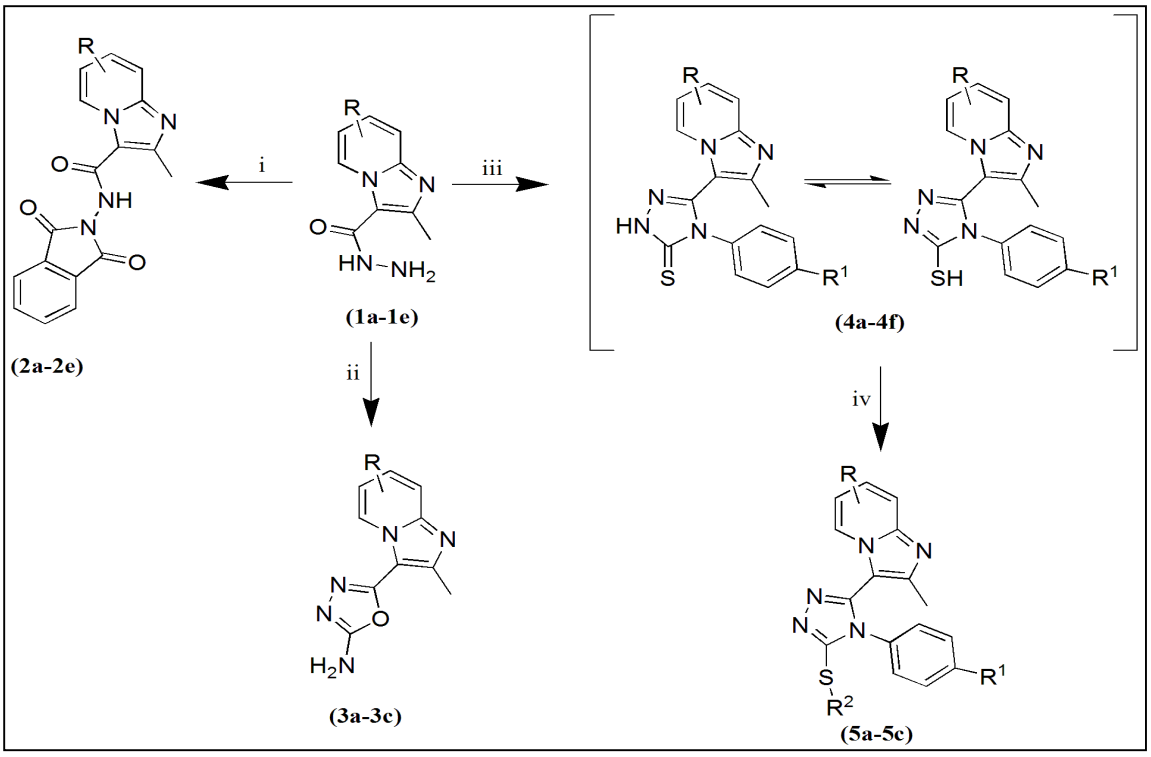
A series of imidazopyridine derivatives containing 1,3,4-oxadiazole (**4a-4j)** were synthesized. The synthetic route for the target compounds were illustrated in **Scheme 1.** As shown, substituted-imidazopyridine-methylimidazo[1,2-a]pyridine-3-carboxylate (**2a-2d**) were synthesized fromsubstituted 2-aminopyridine (**1a-1d)** using ethyl 2-chloroacetoacetate upon 4 hours reflux in dichloromethane solvent. Further, hydrozinolysis with hydrazine hydrate gave the intermediates **3a-3d** in good yield. These were further cyclized with different benzoic acids using phosphorous oxychloride **4a-4j**. The synthesized compounds were characterized by IR, ^{1}HNMR, ^{13}CNMR and Mass spectroscopy, single crystal XRD data were solved for **4c** and **4e,** their Hirshfeld surface analysis was also studied to analyze the percentage of inter-contacts in crystal packing. Further all the compounds were screened for their antimicrobial activities. Among the series, the compound **4f** (R=3-CH_3-pyridine and R^1=3-NO_2-C_6H_5) exhibited both antibacterial and antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* respectively.



Scheme 1: a. Ethyl-2-chloroacetoacetate, DCM, reflux, 4h**; b.** $NH_2NH_2.H_2O$, ethanol, reflux, 10 h**; c.** 4-CH_3/2-Cl/4-Cl/3-OCH_3/CH_2-C_6H_5/3- NO_2benzoicacid, $POCl_3$, reflux, 8 h.

Chapter 3: Synthesis, characterization, single crystal XRD and antimicrobial studies of imidazopyridine derivatives containing oxadiazole,, triazole and isoindoles.

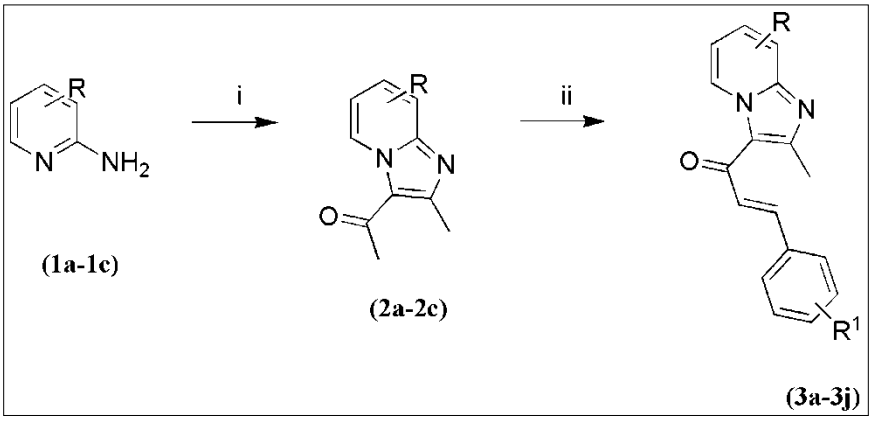
In this chapter, some of the hydrazide intermediates **(1a-1e)** were synthesized as shown in the previous chapter. These were refluxed with phthalic anhydride in the acetic acid media, which yielded the prodcuts **2a-2e**. Also some of the hydrazide cyclized into 2-amino-oxadiazoles using cyanogen bromide **(3a-3c)**. Further from the same intermediates and using phenylisothiocyanates in ethanolic medium under heating condition in the presence of aqueous sodium hydroxide, 1,2,4-triazole-thiones **(4a-4f)** were synthesized. From these some of S-alkylated products were also synthesized **(5a-5c).** All the compounds were well characterized using IR, ^{1}HNMR, ^{13}CNMR and Mass spectroscopy and screened for their antibacterial activities. The compound **3a** (R=3-CH_3-pyridine) and **3c** (R=5-CH_3-pyridine) showed highest percentage of inhibition against *S. aureus* among the synthesized compounds.



Scheme 2: i. Phthalic anhydride, acetic acid, reflux, 6 h**; ii.** CNBr, methanol, reflux, 6h; **iii.** 4-Nitro/4-methyl/4-fluoro/4-methoxyphenylisothiocyanate, ethanol, $NaOH_{(aq.)}$, 8h**; iv.** 2,4-Dichloro/4-nitro-benzylchloride/2-chloro-(4-fluorophenyl)acetamide, ethanol, Et_3N, reflux, 6h;

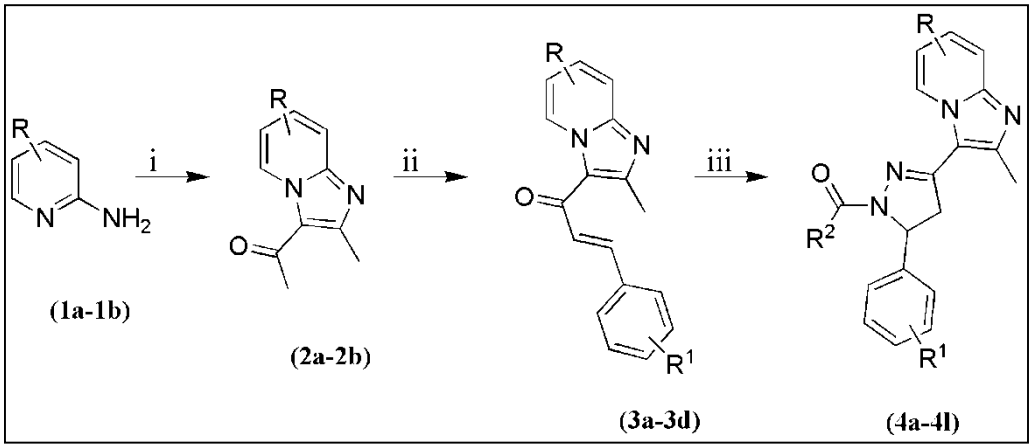
Chapter 4: Synthesis, characterization, single crystal XRD and anticancer studies of some new imidazopyridine incorporated chalcone and pyrazoline derivatives.

This chapter is further subdivided into **Part-A** and **Part-B**. In **Part-A**, the equimolar mixture of methyl substituted 2-aminomethylpyridine **(1a-1c)** and 3-chloro-2,4-pentanedione was refluxed in dichloromethane solvent for about 5 hours. The product formed was extracted and purified which was further utilized to synthesize chalcones **(3a-3j)** by following Clasein-Schimdt react**io**n. All the compounds were well characterized using IR, ^{1}HNMR, ^{13}CNMR, Mass spectroscopyand also using single crystal X-Ray diffraction. All the compounds were screened for their anticancer activity against A549 cell line. Compound **3i** (R=4-CH_3-pyridine, R^1=4-NO_2-C_6H_5) showed significant activity among the series. Further docking and hemocompatibility studies on five most active compounds were done which supported the *in vitro* anticancer result.



Scheme 3: i. 3-chloro-2,4-pentanedione, DCM, reflux, 5 h; **ii.** 2-OH/3-Cl/4-OH/4-NO_2/4-Cl benzaldehyde, $NaOH_{cat}$, ethanol, RT.

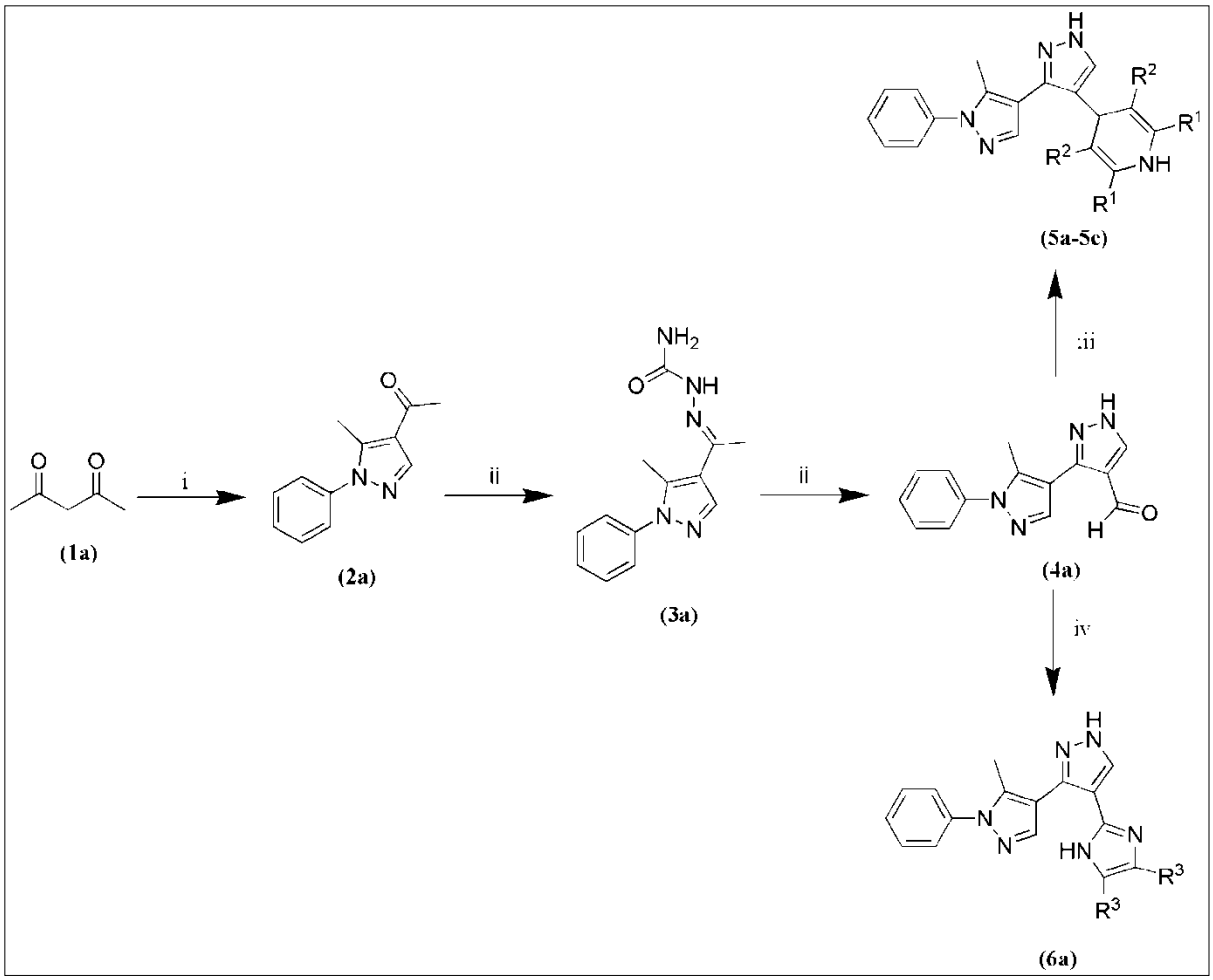
In **Part-B;** Some of the chalcones were further cyclized into their pyrazoline derivatives in the presence of hydrazine hydrate and excess of acetic/propionic/butyric acids under 8 hours reflux condition. The obtained products **(4a-4l)** were characterized by IR, ^{1}HNMR, ^{13}CNMR, Mass spectroscopy with single crystal X-Ray diffraction study. Further all the compounds were studied for their anticancer activity against A549 cell line. The compound **4j** (R= 4-CH_3, R^1= -$COCH_2CH_2CH_3$, R^2= 3-Cl-C_6H_5) showed best activity among the series. The results were further supported by virtual screening and hemocompatibility studies.



Scheme 4:iii. $NH_2NH_2.H_2O$, acetic/propionic/butyric acid, reflux 8 h.

Chapter 5: Synthesis of some new hybrid molecules containing nitrogen heterocycles and their anticancer activities.

The reaction between acetylacetone, *N*,*N*-dimethyldimethoxymethanamine (DMF-DMA) and then with phenylhydrazinewas carried to obtain 1-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)ethan-1-one **(2a)**, which was further refluxed in the presence of semicarbazide and sodiumacetate. The obtained product **(3a)** was further subjected to Vilsmeier-Hack reaction to obtain an aldehyde **(4a).** Further, a series of pyrazole bearing 1,4-dihydropyridine was constructed by the classical Hantzsch condensation **5a-5e** reaction. Then the Debus reaction between **4a** with benzil and ammonium acetate in acetic acid for 6 h,yielded an imidazole derivative **6a** in good yield.The synthesized compounds were confirmed by IR, ^{1}HNMR, ^{13}CNMR and Mass spectroscopy. All the derivatives were screened for their anticancer activity. The compound **5a** (R^1=-CH_3, R^2=-$COOC_2H_5$) showed highest activity among the series.



Scheme 5: i. DMF-DMA, phenylhydrazine, reflux, 4 h; **ii.** Semicarbazide, sodiumacetate, ethanol-water, reflux, 5 h; **iii.** $POCl_3$-DMF, reflux, 7 h; **iv**. EAA/MAA/AA, ammonium acetate, ethanol, reflux, 6 h; **v.**Benzil, ammoniumacetate, Con.HCl, reflux, 6 h.

List of publications:

1. S. Kuthyala, G.K. Nagaraja, S. Sheik, M. Hanumanthappa, M. K. Shankar, Synthesis of imidazo[1,2-a]pyridine-chalcones as potent inhibitors against A549 cell line and their crystal studies. **J. Mol. Struct**. 1177 (2019) 381-390.

2. S. Kuthyala, M. K. Shankar, G. K. Nagaraja, Synthesis, single crystal X-Ray, Hirshfeld and antimicrobial evaluation of some new imidazopyridine nucleus incorporated with oxadiazole scaffold. **Chemistryselect.** 3 (2018) 12894-12899.

3. S. Kuthyala, M. Hanumanthappa, S. M. Kumar, S. Sheik, N. G. Karikannar, A. Prabhu, Crystal, Hirshfeld, ADMET, drug-like and anticancer study of some newly synthesized imidazopyridine containing pyrazoline derivatives. **J. Mol. Struct**. 1197 (2019) 65-72.

Papers presented in National/International Conferences:

1. Sharanya K and G. K. Nagaraja "Synthesis, Characterization and Biological Evaluation of Some Oxadiazole Derivatives", International conference organized by the International Science Congress held at Pacific University, Udaipur Rajasthan; during 8^{th}-9^{th} December 2014. (Poster presentation)

2. Sharanya K, G. K. Nagaraja and Vishma B. L. "Synthesis and Radical Scavenging Activity of Some New Imidazopyridine Derivatives'',International Conference on Science and Technologies; Future Challenges and Solutions (STFCS-2016) held at Mysore University, Mysore; during 8^{th}-9^{th} August 2016.(Poster presentation)

3. Sharanya K and G. K. Nagaraja "An Approach to Synthesize and Characterize Hybrid Molecules of Imidazoles and Pyrazoles",National level Symposium (SYMBIOT) held at Manipal Institute of Technology, Manipal; during 11^{th}-12^{th} August 2017. (Poster presentation)

4. Sharanya K and G. K. Nagaraja "Antioxidant Studies of Some Newly Synthesized Heterocyclic Hybrid Molecules",National Conference on Reaching the Unreached through Science and Technology (NCSTRU-2017) held at Mangalore University, Mangalagangothri during 8^{th}-9^{th} September 2017.(Poster presentation)

5. Sharanya K and G. K. Nagaraja "Synthesis, Characterization and Total Antioxidant Studies of Some New ImidazopyridineChalcone Derivatives", International Conference on Emerging Trends in Chemical Sciences (ICETCS-2017) held at Manipal Institute of Technology, Manipal; during 14^{th}-16^{th}September 2017.(Poster presentation)

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Research student — Research Guide

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